VA[illegible]M215.001AUS PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Hevesi, et al.
Appl. No.	:	09/833,030
Filed	:	April 10, 2001
For	:	METHOD FOR OBTAINING A SURFACE ACTIVATION OF A SOLID SUPPORT FOR BUILDING BIOCHIP MICROARRAYS
Examiner	:	Tran, My-Chau T.

Group Art Unit 1641

#15 Dupp 6-11-03

DECLARATION UNDER 37 C.F.R §1.132

United States Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

1. This Declaration is being submitted to demonstrate the superiority of the method of the present invention in the production of the microarrays that are highly sensitive, reproducible and stable compared to commercially available solid supports produced by various methods of the prior art.

2. I am an inventor on the above-identified patent application and am familiar with the specification and prosecution history.

3. I have extensive experience in the field of the claimed invention as indicated in the attached Curriculum Vitae provided as Exhibit A of the Declaration submitted Sept. 9, 2002.

Appl. No. : 09/833,030
Filed : April 10, 2001

4. The claimed methods allow a greater density of aldehyde groups to be obtained on the surface of the microarray than methods such as those of Weetall and Sundberg, which utilize doubly reactive glutaraldehyde. In such methods, a double-reactive group is present on the glutaraldehyde since one reactive group has to react with the support (containing amino groups) and the other one has to react with the capture probe. However, the use of double-reactive groups limits the reactive conditions since both aldehydes of the same molecule can bind to the amino-groups present on the surface making this molecule inactive for fixation of capture molecule.

The second drawback of the methods using double-reactive glutaraldehydes is that different glutaraldehyde molecules react with each other and polymerize. These drawbacks of the methods in the prior art are difficult to control and they reduce both reduce the density of capture molecules on the surface of the array and reduce the reproducibility of the production of the microarrays.

Indeed, I have found that the methods of the claimed invention produce a higher density of aldehyde groups and allow the grafting of a higher amount of capture molecules per unit area than methods using aldehyde like glutaraldehyde (see Figures 1A, 1B and 2 enclosed herewith).

In figure 1 provided herewith, diaglass slides produced by the claimed method are compared (fixation and hybridization yields) to other aldehyde-derivatized commercially available slides (Super Telechem and Biozyme). The diaglass slides prepared using the claimed methods clearly show superior fixation and hybridization yields.

In figure 2, the same slides were compared in a gene expression experiment where it is crucial to detect both high-copy and low-copy transcripts. Only the diaglass slides were capable of detecting certain low-copy transcripts. The diaglass slides also presented the lowest background.

Appl. No. : 09/833,030
Filed : April 10, 2001

Figure 3 shows that not all direct coupling methods are equally suitable for microarray construction. Figure 3 is a comparative gene expression experiment between the diaglass slides and epoxy-modified glass slides. Even though the epoxy-surface also allows a direct coupling of aminated capture probe, the sensitivity of the diaglass slides is obviously higher.

5. Because of the high concentration of capture molecules per unit area obtained using the claimed methods, the binding of a DNA sequence to its complementary strand on the arrays produced using the claimed methods was found to be a pseudo-first order reaction, which greatly improves the hybridization yield and the quantitation by minimizing the effects of minor differences in capture probe concentration. The pseudo-first order reactions resulting from the high density of capture molecules allows data to be compared between hundreds of different hybridization reactions performed simultaneously on the same array (see the comparison of binding and detection in figures 1-2 enclosed herewith).

In fact, the coefficient of variation from one spot to another on arrays produced using the claimed method was 6%. (See figure 4 provided herewith) In figure 4, biotinylated DNA having an amino-group at its terminus was spotted at 5 concentrations (5, 25, 50, 75, 150 nM) at 9 different locations on an activated glass slide and Cy-3-labeled streptavidin was used for detection. As shown in Figure 4, there was little variation from one location on the microarray to another.

This is an important aspect of the claimed methods, since it is important to have reproducible binding from one location on the array to another in order to allow multiparametric analyses to be performed.

Again, since glutaraldehyde based methods do not provide the high density of aldehyde groups obtained with the present methods, they would not provide the above advantages.

6. In addition, the claimed methods provide a great degree of reproducibility from one array to the next. We have produced about 40,000 functionalized slides for the last 3 years with a

coefficient of variation (CY) lower than 15% for the binding of capture probes on different batches. Such low variability between the surface locations gives the procedure a very unexpected feature: the ability to compare results from two samples analyzed on two different microarrays.

In previous microarray analysis using slides produced by methods of prior art, the variability between microarrays was so high that comparison between two microarrays was nearly impossible. To avoid such limitations, researchers had to hybridize the compared samples on the same array. Each sample had to be labelled with a separate fluorescent dye and the two dyes were measured on the same array. The main drawback of the dual-labeling is that each dye is incorporated at a different level, and further, the quantum yields of different dyes are different.

Because of very high reproducibility and homogeneity of microarray slides produced by the method of the present invention, we can compare any result from one array to the other making the method universal and adaptable to one-dye labeling, thereby avoiding the pitfalls of the dual labeling method. None of the methods described prior to our invention give homogeneous reactive groups on the surface.

7. The claimed methods are compatible with the automated methods used to produce microarrays which employ robotic arrayers which deposit 0.1-10 nanoliter droplets of each DNA probe onto known locations on the solid support. Therefore, the use of an arrayer to build microarrays imposes the following constraints in the linking reaction:

(1) the linking reaction preferably occurs in a fast manner under the process conditions (small volume delivery using an arrayer) so that the reaction is substantially complete at room temperature. However, the reactive group is preferably stable for hours or days because of the long time necessary to build arrays;

(2) the linking reaction preferably achieves a strong link to the substrate in a single step to insure reproducible spotting;

(3) it is also important that the capture probes are effectively bound, preferably by a covalent link to the solid support. The level of binding is preferably the same between various spots on the same array, and between different batches of the arrays.

8. In the methods of Barner cited by the Examiner, N-hydroxylsuccinimide is bound to the support and joined to the capture molecules. However, N-hydroxylsuccinimide is unstable and hydrolyses quickly, rendering it unsuitable for use in microarray fabrication procedures which are optimally conducted using supports having stable reactive moieties.

9. In contrast, the claimed methods are compatible with automated production. In the claimed methods, inert olefinic groups present on the surface of the support are activated into reactive aldehyde groups in a single oxidative step, thereby making the surface ready for microarray production by simple deposition of a solution containing the capture probe with an arrayer on the functionalized surface of the support.

(1) The production of microarrays by the claimed method is very easy and fast (see specification page 4, line 28). Fast coupling reaction is desirable because the small volume dispensed by the arrayer evaporates quickly. The claimed method reduces further handling of the surface with different solutions which would cause loss of homogeneity or modification of the reactive group.

(2) The link between the aldehyde and the capture probe is covalent and direct. Covalent binding allows very stringent washing conditions which are used afterwards for the high specificity of the assay.

10. This demonstrates that the method of the present invention produces microarrays that are clearly superior to those obtained using other methods of generating aldehydes with respect to ease of production, low variability, high sensitivity, and low background.

11. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are

Appl. No. : **09/833,030**
Filed : **April 10, 2001**

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 29/4/2003. By: Remacle. Jose.
[PLEASE FILL IN THE NAME OF THE DECLARANT]

O:\DOCS\MXG\MXG-2458.DOC
042203